National Toxicology Program
Toxicity Report Series
Number 43

NTP Technical Report
on Comparative Toxicity Studies of

o-, *m*-, and *p*-Chloroaniline

(CAS Nos. 95-51-2, 108-42-9, and 106-47-8)

Administered by Gavage
to F344/N Rats and B6C3F$_1$ Mice

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United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Note to the Reader

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- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

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The studies described in this Toxicity Study Report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

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PEER REVIEW

The draft report on the toxicity studies of *o*-chloroaniline, *m*-chloroaniline, and *p*-chloroaniline was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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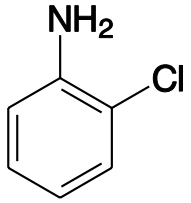
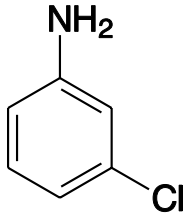
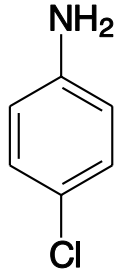
ABSTRACT

	***o*-Chloroaniline**	***m*-Chloroaniline**	***p*-Chloroaniline**
			
CAS Number	95-51-2	108-42-9	106-47-8
Synonyms	*o*-aminochlorobenzene	*m*-aminochlorobenzene	*p*-aminochlorobenzene
	2-chlorobenzenamine	3-chlorobenzenamine	4-chlorobenzenamine
	2-chloroaniline	3-chloroaniline	4-chloroaniline
	2-chlorophenylamine	3-chlorophenylamine	4-chlorophenylamine

Molecular Formula C_6H_6ClN
Molecular Weight 127.57

Chlorinated anilines are used as intermediates in the manufacture of dyes, drugs, and agricultural agents. In comparative 13-week studies conducted to determine the structure-toxicity relationships of *o*-, *m*-, and *p*-chloroaniline, groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were administered 0, 10, 20, 40, 80, or 160 mg *o*- or *m*-chloroaniline per kilogram body weight in dilute hydrochloric acid by gavage. Animals were evaluated for hematology, clinical chemistry, histopathology, and reproductive system effects. Genetic toxicity studies of *o*- and *m*-chloroaniline *in vivo* and *in vitro* were also conducted. The results of the *o*- and *m*-chloroaniline studies were compared to results from the *p*-chloroaniline studies performed previously under similar experimental conditions by the same laboratory; doses in the *p*-chloroaniline studies were 0, 5, 10, 20, 40, and 80 mg/kg for rats and 0, 7.5, 15, 30, 60, and 120 mg/kg for mice.

The hematopoietic system was the target of *o*-, *m*-, and *p*-chloroaniline in rats and mice. Neither the *o*- nor the *p*- isomer had an adverse effect on survival; the death of one female rat in the 160 mg/kg *m*-chloroaniline group during week 12 was possibly secondary to methemoglobinemia. The final mean body weights and weight gains of male rats in the highest dose group in each study and female mice in the 160 mg/kg group in the *o*-chloroaniline study were significantly less than those of the respective controls. Clinical findings of toxicity included a transient bluish discoloration of the genital and footpad regions in rats administered *o*- or *m*-chloroaniline and tremors in rats and mice administered *o*-chloroaniline and in mice administered

m-chloroaniline; these effects occurred primarily in the 80 and 160 mg/kg groups. Methemoglobin concentrations were increased in dosed rats and mice in all studies and resulted in a secondary anemia; the severity of the anemia increased with increasing dose. Microscopic lesions considered related to chemical administration in rats and mice included hemosiderin pigmentation in the bone marrow, kidney, liver, and spleen; hematopoiesis in the liver and spleen; and erythroid cell hyperplasia in the bone marrow. These lesions reflected the response to hemolytic anemia and methemoglobinemia induced by the chloroanilines. A comparative analysis of the results suggests that *p*-chloroaniline is the most potent of the chloroaniline isomers in the induction of methemoglobin formation in rats and mice, followed by *m*-chloroaniline and then by *o*-chloroaniline. This order of potency was also observed for changes in other hematology parameters and in spleen weights, gross and microscopic lesions, and the severity of hemosiderin deposition.

Although the *o*-, *m*-, and *p*- isomers of chloroaniline all exhibit genetic toxicity, the profiles of activity among the three isomers are not identical. *p*-Chloroaniline was mutagenic in all assays in which it was tested, including the *Salmonella* assay, the mouse lymphoma assay, *in vitro* Chinese hamster ovary cell cytogenetics assays, and the *in vivo* mouse bone marrow micronucleus assay; in contrast, *o*- and *m*-chloroaniline gave mixed results among the various assays in which each was tested.

In conclusion, chloroaniline isomers are hematotoxic and have the same pattern of toxicity in rats and mice. Hematotoxicity occurred at all doses in these studies. *p*-Chloroaniline induces the most severe hematotoxic effect, followed by *m*-chloroaniline, then *o*-chloroaniline. Each of the three isomers is more toxic to rats than to mice. *p*-Chloroaniline is clearly genotoxic in various test systems, while the results for the *o*- and *m*- isomers are inconsistent and indicate weak or no genotoxic effects.